spacer regions which are conserved in higher plants would be helpful in persuading him to remove the rejection under §112, first paragraph. The Examiner also indicated that claims 86 and 193 are in condition for allowance.

Request for Continued Examination

A Request for Continued Examination of this case pursuant to 37 C.F.R. §1.114 is filed herewith.

Information Disclosure Statement

An Information Disclosure Statement listing four relevant, non-prior art publications is filed herewith.

Response to the Office Action

Applicant acknowledges the errors in claims 192 and 198. By the foregoing amendment, applicant has replaced "transcriptionally" in line 5 of claim 192 and line 7 of claim 198 with "transcriptional" as suggested by the Examiner.

Applicant acknowledges that the amendments to claims 181 and 183 have not been entered. Applicant further acknowledges that claim 181 has been assigned to Group III.

Applicant acknowledges that claims 184 – 186 have been assigned to Group XV.

Applicant acknowledges that claim 188 has been assigned to Group VII.

Applicant acknowledges that newly submitted claims 200 and 201 are drawn to a non-elected invention and thus have been withdrawn from consideration.

Judicially Created Doctrine of Double Patenting

The Action maintains the judicially created double patenting rejection of claims 2-3, 171, 190-192 and 196-199 over claims 19-23, 25-29, 31 and 34 of U.S. Patent No. 5,932,479. Applicant will address this rejection with a suitable terminal disclaimer once these claims have been found to be otherwise patentable.

Applicant acknowledges the provisional obviousness-type double patenting rejection of claims 2-3. 171, 190-192, and 196-199 over claims 119-120, 124, 132, 140-142, 153-157, 166-167 and 188 of co-pending Application No. 08/972,901. Applicant will address this rejection with a suitable terminal disclaimer if and when Application No. 08/972,901 matures into a patent, and if these claims are determined to be otherwise allowable.

Rejection Under 35 U.S.C. §112, Second Paragraph

The Action rejects claims 2-84, 86-96, 107, 118-119, 122, 168-169, 171-176 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. By the foregoing amendments, applicant has amended claims 2-84, 86-96, 107, 118-119, 122, 168-169, 171-176 as suggested by the Examiner. These rejections have thus been obviated and applicant respectfully requests that they be withdrawn.

Rejection Under 35 U.S.C. §112, First Paragraph

Applicant notes with appreciation the withdrawal-in-part of the rejection under 35 U.S.C. §112, first paragraph.

The Action maintains an enablement rejection on claims 2-3, 171, 190-192, 196-199 on the ground that these claims are broadly drawn to the use of any intergenic spacer region which is highly conserved throughout higher plants and which may be used to facilitate homologous recombination and chloroplast transformation in a multitude of unrelated plant species. Claim 2 has been withdrawn. Claims 3 and 171 have been amended to depend from claim 190.

As discussed during the Interview of September 6, 2001, claim 2 has been withdrawn from consideration. The applicant respectfully disagrees with the conclusion that those skilled in the art are invited to conduct undue experimentation. The specification provides methods to identify appropriate untranscribed intergenic spacer sequences in plants which are useful in the invention (pgs. 27-28). One method calls for isolating plastid genomic DNA, carrying out hybridization with a radioactive labeled probe of a known spacer, detecting and isolating plastid sequences which exhibit that desired degree of homology with the probe. The desired degree of homology is 90% - 100% when hybridization is under stringent conditions and 60% to 100% when under non-stringent conditions. The specification also teaches that a lower degree of hybridization – defined as about 60% - is acceptable if one's requirement of homologous recombination is more permissive. Another method to identify highly conserved regions in the plastid genome is the utilization of the BLAST program. These techniques required are known to those in the art and the parameters of suitable regions are specifically elucidated. Given the reasonable amount of guidance in the specification, skilled artisans are not required to undergo undue explanation.

Further, evidence shows that other spacer sequences appropriate for vector construction exists. In works published subsequent to the filing of this application, at least three regions have been found to be suitable regions. These include the regions as shown in the Sidorov,

Kavanaugh, and Ruf publications. These publications have been provided in an Information Disclosure Statement filed herewith. The existence of these regions strongly suggests that other suitable spacer sequences exists and may be located by those skilled in the art using the method taught in the specification.

The Examiner has also rejected claims 190-191 and 196-197 under 35 U.S.C. §112, first paragraph. The Examiner views the specification as not providing enablement to those skilled in the art to use any intergenic spacer region for the integration of heterologous DNA into transcriptionally active regions of the chloroplast genome. The Examiner states that:

> The specification only provides guidance for the insertion of heterologous DNA into an intergenic region of the chloroplast genome, due to the introduction of an expression vector comprising a heterologous DNA segment flanked by a portion of an intergenic region. By definition, an intergenic region is transcriptionally silent, since there are no promoters present between two genes. The specification provides no guidance for the insertion of any heterologous DNA into a transcriptionally active region of the chloroplast genome, particularly when intergenic spacer regions are used as the flanking regions of the heterologous DNA construct. In contrast, the claims are broadly drawn to any intergenic spacer region or any other flanking region of any sequence and from any source, and the introduction into a multitude of transcriptionally active regions of the chloroplast genome of a multitude of unrelated plant species.

Applicant maintains that the specification fully enables one skilled in the art to practice the invention. As stated above, those skilled in the art, when viewing this disclosure, will understand the method of isolating suitable regions for constructing a vector of the invention. The applicant also respectfully disagrees with the Examiner's statement that intergenic regions are by definition transcriptionally silent. It is known in the art that chloroplast terminators are inefficient in transcription termination. Thus transcription is not terminated even in the presence of a terminator. The Examiner's attention is respectfully drawn to a publication by Staub and

Maliga entitled "Expression of a chimeric *uidA* gene indicates that polycistronic mRNAs are efficiently translated in tobacco plastids" (1995). Therein, a promoterless *uidA* reporter gene (GUS) when inserted into a spacer region produced a polycistronic transcript and the reporter gene product was observed in large amounts even though the report gene was promoterless. It is therefore asserted that intergenic regions may be transcriptionally actively and the specification's disclosure is enabling.

Prior Art Rejections

Zoubenko et al

The Action rejects claims 2-3, 171, 192 and 198-199 as anticipated by Zoubenko et al. During the Interview of September 6, 2001, claim 2 was withdrawn from consideration. In addition, the Examiner indicated that claims 198 and 199 are free of prior art. Claims 3 and 171 have been amended to depend from claim 190. Only claim 192 remains rejected over Zoubenko. Claim 192 has been amended to call for use of flanking regions of plastid DNA that originate from a different species of plant than the target plant.

In the Action, the Examiner regards the intergenic region to be "inherently conserved to some degree, absent any evidence to the contrary" and therefore, Zoubenko et al.'s failure to teach the use of such a region is immaterial.

The Examiner's attention is respectfully drawn to the section entitled "Prior Art Concepts of the Intergenic Spacer Region" on page 7 of the specification. This section discloses that the relevant prior art teaches that:

> "...the sequences flanking functional genes, i.e. the spacer regions between coding regions typically are not conserved. The accepted

> dogma for lack of conservation, and thus the low degree of homology between species of spacer regions, is that the spacer regions typically do not perform essential functions. Therefore, there is little, if any, selective pressure to conserve the sequence of spacer regions between species. The sequence of the spacer regions may be altered without undesirable effects.
>
> Stummann et al., 1988, disclose that the gene order of the ribosomal RNA operon of the chloroplast genome is the same between different species of plants, including tobacco, maize, and a liverwort, *Marchantia*, and that the coding sequences of this operon are highly homologous. Stummann also discloses that the interspecies homology of the operon is less that the interspecies homology of the gene coding regions. This is consistent with the lack of conservation of spacer region; and suggests that the interspecies homology of spacer regions in the ribosomal RNA operon is relatively low."

In contrast to the prior art, the present invention teaches that there are spacer regions which are highly conserved between different plants. The specification also teaches methods to identify these spacer regions (pg. 27-28). Applicant submits that this is an important, novel concept not taught in Zoubenko et al. and contra to existing accepted dogma.

BIOTECHNICA

The Examiner has also rejected claims 2-3 as anticipated by BIOTECHNICA. Claim 2 has been withdrawn from consideration. Claim 3 has been amended to depend from claim 190. Applicant asserts that, as a result of these amendments, the rejection over BIOTECHNICA has been obviated.

Staub et al

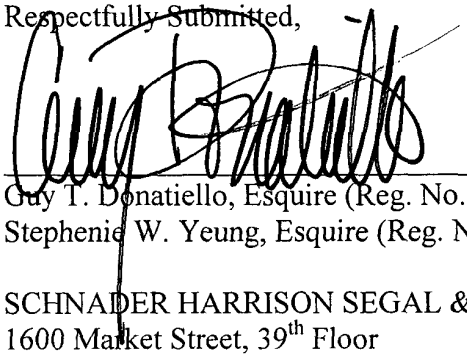
The Examiner also rejected claims 2-3, 171, 192 and 198-199 as anticipated by Staub et al. During the Interview of September 6, 2001, the Examiner indicated that claims 198 and 199 are free of prior art. Claim 2 has been withdrawn from consideration. Claims 3 and 171 now depend from claim 190. Only claim 192 remains rejected over Staub. Claim 192 has been amended to call for use of flanking regions of plastid DNA that originate from a different species of plant than the target plant.

Applicant respectfully traverses the rejection. Neither publication by Staub anticipate the present invention. The 1993 publication teaches the transformation of tobacco with a vector containing flanking sequences derived from tobacco. There is no teaching of the use of the homology region to transform plant species other than tobacco. The 1992 publication is likewise limited to tobacco transformation using a vector containing targeting sequence derived from tobacco. Nor does either of the publication teach insertion of genes into transcriptionally active regions. Since neither publication shows transformation of plant species using sequence derived from another plant species, applicant submits that the Staub publication fails to teach an important aspect of the invention and should be withdrawn as prior art.

Conclusion

For the foregoing reasons, Applicant respectfully requests favorable consideration of this application.

Respectfully Submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES TO THE CLAIMS

Amend the following claims:

2. Cancelled.

3. (Once Amended) The vector of claim ~~2~~ 190 which comprises a heterologous nucleotide sequence coding for a selectable phenotype.

86. (Twice Amended) A process for stably transforming a target higher plant species which comprises introducing an integration and expression universal vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent to stably transform the chloroplast of higher plants and comprising an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest, and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequence to provide expression of the coding sequence in the chloroplast genome of the target higher plant, a heterologous nucleotide sequence coding for a selectable phenotype, and flanking each side of the expression cassette, chloroplast DNA sequences of a higher plant which comprise each one a portion of the intergenic spacer 2 region between the $tRNA^{Ile}$ and the $tRNA^{Ala}$ genes of the chloroplast genome, said chloroplast sequences conserved in all higher plants and competent of undergoing homologous recombination with complementary spacer 2 sequences of heterologous target plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the complementary spacer 2 sequences of the target plant chloroplast genome.

171. (Once Amended) The universal integration and expression vector of claim ~~2~~ 190 which does not include a transposon.

190. (Once Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, chloroplast DNA sequences which originate from a plant species ~~the same as or~~ different from the target plant, said chloroplast sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which chloroplast sequences are also competent of undergoing homologous recombination with said complementary sequences, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated by said homologous recombination of the flanking sequences with the complementary sequences in the target chloroplast genome, and wherein said stable integration is not directed into a transcriptionally inactive region of the chloroplast genome.

191.(Once Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA

sequences which originate from a plant species ~~the same as or~~ different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into ~~including~~ a transcriptionally active region of the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome.

192.(Once Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences including a ~~transcriptionally~~ transcription termination region to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species ~~the same as or~~ different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding

sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences

193. (Once Amended) A universal integration and expression vector competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking chloroplast DNA sequences each one a portion of a synthetic spacer 2 region between the $tRNA^{Ile}$ and $tRNA^{Ala}$, said chloroplast sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which chloroplast sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome.

194. (Once Amended) The process of claim 86 wherein the flanking ~~sequence~~ sequences ~~which~~ originate from ~~an original~~ other than the target plant and comprise, each one a portion of the intergenic spacer 2 region between the $tRNA^{Ile}$ and the $tRNA^{Ala}$ genes of the chloroplast genome, whereby double homologous recombination with the conserved spacer 2 region in the target chloroplast genome is facilitated.

196. (Once Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species ~~the same as or~~ different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome, of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome, and wherein said stable integration is not directed into a transcriptionally inactive region of the chloroplast genome.

197. (Once Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which

comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species ~~the same as or~~ different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into ~~including~~ a transcriptionally active region of the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome.

198. (Once Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence including a ~~transcriptionally~~ transcription termination region in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA

sequences which originate from a plant species ~~the same as or~~ different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant and which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome.

199. (Once Amended) A process for stably transforming higher target plant species which comprises introducing a universal integration and expression vector into the chloroplast genome of the target plant species and allowing the transformed plant to grow, the vector being competent for stably transforming the chloroplast genome of higher plant species which comprises an expression cassette which comprises, operably joined, a heterologous DNA sequence coding for a peptide of interest and control sequences positioned upstream from the 5' and downstream from the 3' ends of the coding sequences to provide expression of the coding sequence in the chloroplast genome of a target higher plant, and flanking each side of the expression cassette, flanking DNA sequences which originate from a plant species ~~the same as or~~ different from the target plant, said flanking sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which flanking sequences are also competent of undergoing homologous recombination with said complementary sequences of the target plant which are homologous to a spacer sequence of the target chloroplast genome, which sequence is conserved in the chloroplast genome of different

plant species, whereby stable integration of the heterologous coding sequence into the chloroplast genome of the target plant is facilitated through homologous recombination of the flanking sequences with the homologous sequences in the target chloroplast genome and the vector does not include a transposon.

202. (Newly added) A vector for transformation of crop plants, which comprises a first flanking sequence, a DNA sequence coding for a transcription origin, a promoter, a heterologous DNA sequence encoding a gene of interest, a DNA sequence encoding a selectable marker, a terminator, a second flanking sequence, wherein said first and second flanking sequences are sequences derived from a plastid genome which are highly conserved among crop plants and which are not derived from a plastid of a plant to be transformed and which facilitate stable transformation of the plant to be transformed through homologous recombination of the first and second flanking sequences with complementary sequences of a plastid of the plant to be transformed.

203. (Newly added) A vector of claim 202, wherein the first and second flanking sequences are derived from tobacco.

204. (Newly added) A vector of claim 202, wherein the first and second flanking sequences are derived from *Solanum nigrum*.

205. (Newly added) A vector of claim 202, wherein the crop plant is selected from a group consisting of cotton, maize, rice, barley, lettuce, and soybeans.

206. (Newly added) A vector for transformation of crop plants, which comprises a first flanking sequence, a DNA sequence coding for a transcription origin, a promoter, a heterologous DNA sequence encoding a gene of interest, a DNA sequence encoding a selectable marker, a terminator, a second flanking sequence, wherein said first and second flanking sequences are

sequences derived from a plastid genome which are highly conserved among crop plants and which are not derived from a plastid of a plant to be transformed and which facilitate stable transformation of the plant to be transformed through homologous recombination of the first and second flanking sequences with complementary sequences of a plastid of the plant to be transformed, wherein homology between the flanking sequence is between 60% and 100%.

207. (Newly added) A vector of claim 206, wherein the first and second flanking sequences are derived from tobacco.

208. (Newly added) A vector of claim 206, wherein the first and second flanking sequences are derived from *Solanum nigrum*.

209. (Newly added) A vector of claim 206, wherein the crop plant is selected from a group consisting of cotton, maize, rice, barley, lettuce, and soybeans.

210. (Newly added) A stably transformed crop plant and progeny thereof, wherein a plastid of said crop plant has been transformed by a vector which comprises a first flanking sequence, a DNA sequence coding for a transcription origin, a promoter, a heterologous DNA sequence encoding a gene of interest, a DNA sequence encoding a selectable marker, a terminator, a second flanking sequence, wherein said first and second flanking sequences are sequences derived from a plastid genome which are highly conserved among crop plants and which are not derived from a plastid of a plant to be transformed and which facilitate stable transformation of the plant to be transformed through homologous recombination of the first and second flanking sequences with complementary sequences of a plastid of the plant to be transformed.

211. (Newly added) A stably transformed plant of claim 210, wherein homology between the flanking sequence is between 60% and 100%.

212. (Newly added) A process for stably transforming a crop plant species, comprising the steps of:

providing a crop plant species;

introducing a vector into the chloroplast genome of said crop plant species by bombardment; and

allowing said crop plant species, now transformed, to grow;

wherein said vector comprises a first flanking sequence, a DNA sequence coding for a transcription origin, a promoter, a heterologous DNA sequence encoding a gene of interest, a DNA sequence encoding a selectable marker, a terminator, a second flanking sequence, wherein said first and second flanking sequences are sequences derived from a plastid genome which are highly conserved among crop plants and which are not derived from a plastid of a plant to be transformed and which facilitate stable transformation of the plant to be transformed through homologous recombination of the first and second flanking sequences with complementary sequences of a plastid of the plant to be transformed.

213. (Newly added) A process for stably transforming a crop plant species, comprising the steps of:

providing a crop plant species;

introducing a vector into the chloroplast genome of said crop plant species by bombardment; and

allowing said crop plant species, now transformed, to grow;

wherein said vector comprises a first flanking sequence, a DNA sequence coding for a transcription origin, a promoter, a heterologous DNA sequence encoding a gene of interest, a DNA sequence encoding a selectable marker, a terminator, a second flanking sequence, wherein said first and second flanking sequences are sequences derived from a plastid genome which are highly conserved among crop plants and which are not derived from a plastid of a plant to be

transformed and which facilitate stable transformation of the plant to be transformed through homologous recombination of the first and second flanking sequences with complementary sequences of a plastid of the plant to be transformed, wherein homology between the flanking sequence is between 60% and 100%.